Leptin concentrations in serum from a randomly recruited sample of 50- to 80-year-old men and women: positive association with plasma insulin-like growth factors (IGFs) and IGF-binding protein-3 in lean, but not in obese, individuals

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Abstract

Objective: The GH/IGF axis is thought to play an important role in the regulation of body composition throughout life. Changes in body fat stores also affect the activity of the GH/IGF axis, but the mechanisms whereby body fat status is signaled to the GH/IGF axis are poorly understood. The newly discovered protein leptin is exclusively produced by adipocytes, and circulating concentrations of leptin closely reflect body fat stores.

Design: We here examined whether leptin might be associated with the activity of the GH/IGF axis in a population-based sample.

Patients and methods: Circulating concentrations of leptin, IGF-I, IGF-II, and insulin-like growth factor-binding protein-3 (IGFBP-3) were measured in a population-based sample of 50- to 80-year-old men ($n=217$) and women ($n=198$) by specific RIA.

Results: All three IGF components were significantly positively correlated with leptin in lean women (body mass index (BMI) $<25 \text{ kg/m}^2$). IGF-II was also positively correlated with leptin in lean men, and positive correlation of leptin with IGF-I in lean men was of borderline statistical significance. In contrast, no correlation was observed in moderately overweight (BMI $25\text{--}30 \text{ kg/m}^2$) and obese individuals (BMI $>30 \text{ kg/m}^2$).

Conclusion: Our study shows that serum leptin concentrations are significantly associated with circulating IGF components in lean elderly subjects. The precise mechanism of this interaction between leptin and the GH/IGF system remains to be determined.

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Introduction

Aging is associated with multiple alterations in energy balance and body composition (1). There are multiple causes for these age-related changes in body composition, but part of them may be explained by the gradual decline in the activity of the growth hormone (GH)/insulin-like growth factor (IGF) axis (2). GH is both anabolic and lipolytic (3–5). Fat mass is increased and lean body mass decreased in GH-deficient humans, and these changes are reversed by GH (3–5). Replacement of GH in older men has been shown to reverse some of the body composition changes associated with aging (2, 6–8). Most of the anabolic and lipolytic effects of GH are thought to be mediated by the IGFs (8). There are two isoforms of IGF, IGF-I and IGF-II. In the circulation the IGFs are predominantly bound in a 150 kDa

complex which consists of IGF-I or IGF-II, IGF-binding protein-3 (IGFBP-3), and an acid-labile subunit (8, 9). GH is the major regulator of IGF-I and IGFBP-3, and it also indirectly determines IGF-II activity due to the close association of IGF-II to IGFBP-3. In addition, insulin, thyroid hormones and supply of dietary energy and protein may directly regulate the circulating levels of the IGFs and of IGFBP-3 (8, 9), suggesting that the IGF system plays an important role in the regulation of fuel metabolism beyond its association with GH. However, the mechanisms whereby GH and the IGF systems respond to changes in body fat and body composition are still poorly understood.

Leptin is a recently discovered protein that is exclusively synthesized in adipose tissue and is encoded by the *ob* gene (10). It decreases food intake by causing satiety and promoting energy consumption (10).

Non-functional mutations of murine leptin result in an inherited form of obesity in ob/ob mice (10). Interestingly, human plasma leptin concentrations are subject to circadian changes (11) that parallel those observed for the secretion of GH (12). Moreover, the effects of leptin on body composition appear to be mediated by specific receptors in the hypothalamus (13–15), a brain region that is critical for regulation and circadian rhythm of GH activity. In order to examine whether leptin may provide a link between body fat stores and the activity of the GH/IGF axis, we here examined the association of serum leptin with circulating IGF components in a population-based sample of 50- to 80-year-old men and women, whose concentrations of circulating IGFs have been recently reported (16). Since some experimental data (17) suggest a relative leptin resistance in obese individuals, we also addressed the question whether a possible association between the GH/IGF axis and leptin might depend on the individuals' body mass indices (BMI).

Subjects and methods

Study population

Serum samples were obtained as part of the European Vertebral Osteoporosis Study (18). An approximately 30% random sample of the birth cohorts 1910–1940 was recruited in Eppelheim, a suburb of Heidelberg, by written invitation between January 1992 and March 1993. The study included 1072 men and women, aged 50–79 years at baseline. Random sampling within age and sex strata was used to obtain equal numbers of men and women in each 5-year age group. Of the subjects, 996 were contactable and eligible. Of these, 58% ($n = 580$), 297 men and 283 women, agreed to participate. Non-fasting blood samples were drawn between 0800 and 1130 h, immediately separated and stored at -80°C until assayed. Sufficient serum samples for the measurement of all IGF components and leptin were available in 217 men and 198 women. Standardized measures of weight and height were obtained with participants in light clothing and shoes removed. BMI (weight (kg)/height (m^2)) and the waist-to-hip-ratio (WHR) were used as measures of obesity. The study was approved by the ethics committee on clinical investigations at the University of Heidelberg, Medical Center, and participants provided written consent.

Measurement of serum leptin

Serum leptin concentrations were determined by a specific RIA which has been described in detail elsewhere (19). In brief, recombinant human leptin (a kind gift of Dr Heiman, Eli Lilly Research Laboratories, Indianapolis, IN, USA) was used for the production of

antiserum in rabbits, and for the preparation of tracer, by the Chloramine T method, and of standards. The assay buffer was composed of 0.05 mol/l sodium phosphate, pH 7.4, 0.1 mol/l NaCl, 0.05% (w:v) NaN_3 , 0.1% (v:v) gelatine from teleost fish (Sigma, München, Germany), and 0.1% (v:v) Triton X-100. The assay volume was 0.3 ml. After incubation at room temperature overnight, bound and unbound tracer were separated by a second antibody technique. Maximal tracer binding was 37–45% and half-maximal binding occurred at $0.9 \mu\text{g/l}$ unlabeled leptin. Excellent parallelism was obtained with serial dilutions of human serum, and spiking experiments with 0.1 ng per tube yielded a recovery of $97.0 \pm 2.1\%$. Sensitivity was $0.03 \mu\text{g/l}$ and intra- and interassay coefficients of variation (CV) were 0.8% and 8.5% respectively.

Measurement of plasma IGF components

Measurement of plasma IGF components was carried out as described previously (16). IGF-I was measured by RIA, using a polyclonal rabbit antibody specific for human IGF-I (Mediagnost, Tübingen, Germany) and recombinant human IGF-I (GroPep, Adelaide, Australia) as tracer. IGFBP artifacts were avoided by initial dissociation of IGF-I from IGFBPs using an acid buffer (20). Reassociation was then blocked by IGFBP saturation through the addition of excess IGF-II (Mediagnost). Cross-reactivity with IGF-II was less than 0.05%. IGF-II was measured by RIA using an anti-IGF-II antibody obtained by immunization of rabbits using a synthetic peptide IGF-II. Cross-reactivity of the IGF-II antibody with IGF-I was less than 0.05%. IGFBP artifacts were avoided in the same manner as for IGF-I assay, except that excess IGF-I was used to block reassociation. Inter-assay CV values for both assays were 5–7% at a sensitivity of 0.1 ng/ml. IGFBP-3 was measured using a commercial RIA from Biomerieux (Nürtingen, Germany). Interassay CV was 8.2% at a sensitivity level of 0.06 ng/ml.

Statistical analysis

Simple and partial Pearson correlations were used to assess the strength of association of serum leptin concentrations to BMI or IGF-related parameters. Means of continuous variables were compared between groups using Student's *t*-test or analysis of variance (general linear model) for unbalanced designs as appropriate. Since leptin distribution significantly deviated from a normal distribution, measurements were log transformed for analysis. Statistical significance was set at a probability level of $\alpha = 0.05$ based on two-sided tests. All analyses and descriptive statistics were performed using the Statistical Analysis System software program (SAS Institute, Cary, NC, USA).

Table 1 Distribution of serum leptin, circulating IGF components, and BMI in 198 women, aged 50–80 years. Values are median (90 percentile range).

	All (n = 198)	BMI < 25 kg/m ² (n = 85)	BMI 25–30 kg/m ² (n = 72)	BMI > 30 kg/m ² (n = 41)
Leptin (ng/ml)	10.9 (2.6–19.6)	6.9 (1.7–19.6)	13.6* (4.2–27.1)	23.7* (11.4–46.1)
IGF-I (nmol/l)	18.0 (10.2–29.5)	18.0 (9.3–27.5)	17.7 (11.8–30.0)	18.7 (11.5–27.5)
IGF-II (nmol/l)	137.9 (98.6–178.4)	127.1 (98.8–162.4)	134.3 (91.8–173.0)	138.6* (111.2–186.5)
IGFBP-3 (nmol/l)	109.2 (68.6–154.7)	106.1 (65.1–141.1)	107.8 (63.7–154.7)	123.4* (82.4–157.9)
BMI (kg/m ²)	25.6 (20.3–34.4)	23.1 (18.7–24.7)	26.3 (25.3–29.4)	32.0 (30.1–38.9)
Age (years)	64.0 (53.0–80.0)	64.0 (53.0–80.0)	63.0 (53.0–80.0)	64.5 (55.5–73.5)
Albumin (g/l)	46.0 (41.0–51.0)	46.0 (40.0–51.0)	46.0 (42.0–50.0)	46.0 (41.0–51.0)

Significant differences of moderately overweight or obese women compared with lean women are marked with * ($P < 0.05$, general linear model).

Results

Associations of leptin and IGF components with BMI

The distribution of leptin and circulating IGF-I, IGF-II, and IGFBP-3 in women and men are presented in Tables 1 and 2. Individuals were divided into three classes, comparable in mean age, according to their BMI: lean individuals with a BMI below 25 kg/m², moderately overweight individuals with a BMI between 25 and 30 kg/m², and obese individuals with a BMI exceeding 30 kg/m². Leptin concentrations were approximately 2.5-fold higher in women compared with men in all three BMI classes. In both sexes, leptin levels were approximately 2-fold higher in the moderately overweight individuals and 3-fold higher in the obese individuals compared with the lean individuals. There

was a strong correlation between leptin concentrations and BMI in men ($r = +0.60$, $P < 0.0001$) and in women ($r = +0.72$, $P < 0.0001$). Similar, albeit weaker, associations were also observed between leptin and WHR in both men ($r = +0.43$, $P < 0.0001$) and women ($r = +0.49$, $P < 0.0001$). These associations were significant in all three BMI groups (data not shown). Plasma IGF-I concentrations did not significantly differ between obese and non-obese subjects, but IGF-II and IGFBP-3 concentrations were slightly higher in obese women (Tables 1 and 2). All three circulating IGF components were strongly intercorrelated with each other (IGF-I vs IGF-II: $r = +0.44$, $P < 0.0001$; IGF-I vs IGFBP-3: $r = +0.60$, $P < 0.0001$; IGF-II vs IGFBP-3: $r = +0.87$, $P < 0.0001$). Inverse correlations with age were observed for all three IGF components (IGF-I: $r = -0.15$, $P = 0.003$; IGF-II: $r = -0.16$, $P = 0.001$; IGFBP-3: $r = -0.19$, $P = 0.0001$), as has been reported previously (16). In

Table 2 Distribution of serum leptin, circulating IGF components, and BMI in 217 men, aged 50–80 years. Values are median (90 percentile range).

	All (n = 217)	BMI < 25 kg/m ² (n = 55)	BMI 25–30 kg/m ² (n = 121)	BMI > 30 kg/m ² (n = 41)
Leptin (ng/ml)	4.4 (1.3–12.8)	2.5 (0.9–7.5)	4.4* (1.7–9.5)	8.0* (3.5–14.4)
IGF-I (nmol/l)	18.7 (11.2–30.4)	18.8 (11.7–29.6)	19.6 (11.5–31.4)	16.4 (10.4–28.1)
IGF-II (nmol/l)	115.6 (89.6–152.8)	113.0 (93.0–160.3)	116.8 (87.5–151.6)	112.2 (89.6–160.9)
IGFBP-3 (nmol/l)	96.3 (55.3–136.5)	92.6 (54.3–138.3)	98.0 (58.1–136.5)	97.0 (51.8–136.5)
BMI (kg/m ²)	27.0 (22.3–33.8)	23.7 (20.4–24.7)	27.1 (25.3–29.2)	32.1 (30.4–35.8)
Age (years)	60.0 (52.0–80.0)	68.0 (52.0–79.0)	66.0 (53.0–80.0)	63.0 (53.0–77.0)
Albumin (g/l)	46.0 (42.0–50.0)	46.0 (40.0–51.0)	46.0 (42.0–50.0)	46.0 (43.0–50.0)

Significant differences of moderately overweight or obese men compared with lean men are marked with * ($P < 0.05$, general linear model).

Table 3 Correlation between serum leptin and circulating IGF components in 50- to 80-year-old women.

	All (<i>n</i> = 198)		BMI < 25 kg/m ² (<i>n</i> = 85)		BMI 25–30 kg/m ² (<i>n</i> = 72)		BMI > 30 kg/m ² (<i>n</i> = 41)	
	Simple	/BMI	Simple	/BMI	Simple	/BMI	Simple	/BMI
IGF-I	+0.09	+0.11	+0.21 ^a	+0.17	−0.06	+0.06	−0.03	−0.01
IGF-II	+0.28 ^d	+0.19 ^c	+0.35 ^b	+0.31 ^b	+0.02	+0.14	−0.01	+0.007
IGFBP-3	+0.24 ^c	+0.15 ^a	+0.32 ^b	+0.28 ^a	−0.002	+0.12	−0.17	−0.15

Pearson correlation coefficients for simple correlation, and partial correlation after adjustment for BMI. Significant associations of leptin with IGF components: ^a *P* < 0.05, ^b *P* < 0.01, ^c *P* < 0.001, ^d *P* < 0.0001.

contrast, leptin concentrations were unchanged with age in men and women (men: *r* = +0.06, *P* = 0.37; women: *r* = +0.02, *P* = 0.78). The ratio of leptin to BMI was unchanged with aging (men: *r* = +0.07, *P* = 0.30; women: *r* = +0.05, *P* = 0.49). This was also true within all three BMI classes.

Association of leptin with circulating IGF components

In women, but not in men, a significant positive correlation was observed between the circulating concentrations of IGF-II and IGFBP-3 on the one hand and serum leptin concentrations on the other hand (Tables 3 and 4). Stratification for BMI revealed that these associations only occurred in lean women. Lean women also exhibited a positive correlation between serum leptin and circulating IGF-I (Table 3). Analyzed by a linear regression model, leptin concentrations explained between 4% and 12% of the variability of the IGF components in this BMI class. A similar association existed between IGF-II and leptin in lean men, and the correlation between leptin and IGF-I was of borderline statistical significance (Table 4). Most of the associations of leptin with these IGF components only slightly decreased in strength after adjustment for BMI or WHR. In contrast, there were no significant associations between leptin and any of the IGF parameters in moderately overweight or obese subjects (Tables 3 and 4). This lack of association persisted upon adjustment for BMI (Tables 3 and 4) or WHR (data not shown) and was still apparent when analysis was performed for all subjects with a BMI greater than 25 kg/m² (data not shown). Increases in serum creatinine tended to be

associated with slightly higher leptin levels (*r* = +0.13, *P* = 0.05 for both sexes), but adjustment for differences in serum creatinine did not substantially alter the correlation between leptin and any of the IGF parameters (data not shown). A positive correlation between IGF-II and IGFBP-3 and leptin was also observed in individuals with WHR values <1 as a measure of abdominal obesity, but not in individuals with WHR values ≥1 (data not shown).

Discussion

The relationship between leptin and the principal regulators of human body composition and fuel metabolism is still largely unknown. Using a well-defined population sample of 50- to 80-year-old randomly recruited ambulatory men and women, we here examined the relationship between serum leptin levels and circulating components of the GH/IGF axis.

Our data show that there are considerable gender- and weight-related differences between the concentrations of leptin and circulating IGF-I, IGF-II, and IGFBP-3. In accordance with previous findings (21–23), leptin was strongly associated with BMI in both sexes. In contrast, increases in BMI resulted in only moderate increases in IGF-II and IGFBP-3 in women, and failed to be associated with any changes in IGF-I in men. Furthermore, all three IGF components significantly declined with age, but leptin concentrations remained unchanged with aging in both sexes. Finally, as has been shown in previous studies (10), leptin concentrations were about 2.5-fold higher in women compared with men, irrespective of BMI. In contrast, the plasma concentrations of IGF-II and IGFBP-3 were only slightly

Table 4 Correlation between serum leptin and circulating IGF components in 50- to 80-year-old men.

	All (<i>n</i> = 217)		BMI < 25 kg/m ² (<i>n</i> = 55)		BMI 25–30 kg/m ² (<i>n</i> = 121)		BMI > 30 kg/m ² (<i>n</i> = 41)	
	Simple	/BMI	Simple	/BMI	Simple	/BMI	Simple	/BMI
IGF-I	−0.06	−0.01	+0.24 ^a	+0.23	−0.05	−0.13	−0.17	−0.19
IGF-II	+0.08	+0.11	+0.44 ^b	+0.43 ^b	−0.05	−0.10	+0.09	+0.12
IGFBP-3	+0.02	−0.05	+0.19	+0.16	−0.07	−0.16	−0.11	−0.15

Pearson correlation coefficients for simple correlation, and partial correlation after adjustment for BMI. Significant or borderline significant associations of leptin with IGF components: ^a *P* = 0.09, ^b *P* < 0.01.

higher in women compared with age-matched men, and there was no difference in circulating IGF-I concentrations between both sexes.

Despite these obvious differences between leptin and the IGF-system, our study demonstrates that in lean elderly individuals, plasma IGF-I, IGF-II, and IGFBP-3 concentrations proportionally increase with increasing leptin concentrations. The patients in our study were healthy ambulatory 50- to 80-year-old men and women without malnutrition or cachexia, which is also reflected by the normal serum albumin and IGF-I values in all BMI groups. A positive correlation between IGF-I and leptin has also been recently reported by Grinspoon *et al.* (24) in young women with anorexia nervosa. Interestingly, the association in these women remained significant in a multivariate regression model even when the effects of weight, fat mass, and caloric intake were accounted for. However, due to the large deviations in caloric intake in these patients, these findings are difficult to interpret, and population-based data are necessary to see whether there is a similar relationship between leptin and the IGF system as observed in our study in younger individuals.

All three IGF components are closely interrelated to each other and to GH activity (9). The strongest associations with serum leptin in the present study were observed for IGF-II, which is linked to GH activity through its tight association with GH-regulated IGFBP-3. Interestingly, despite this close association between IGFBP-3 and IGF-II, IGFBP-3 failed to be significantly associated with leptin in lean men. The reason for this is currently unclear. It is possible that the weak association in men may have simply occurred by chance. However, it remains a possibility that the observed gender differences are real, and that leptin may have differential effects on IGFBP-3 regulation in men and women by a yet unexplained mechanism.

The correlative nature of our data does not allow us to draw any definitive conclusions with respect to the mechanisms of the observed associations. However, apart from a possible direct effect of leptin on the circulating IGF components, there is some evidence which points to a potential role of leptin in GH secretion. Since the reduction in fat mass by leptin is not solely explained by its hypothalamic effects on food intake (25), it is possible that GH secretion may be one of the targets of leptin. Indeed, while our work was in progress, Carro *et al.* (26) found a marked reduction of GH secretion in rats after administration of inhibiting antibodies to leptin. One of the possible mechanisms by which leptin might affect GH activity may be through the suppression of neuropeptide Y (NPY) expression in the arcuate nucleus of the hypothalamus (13). NPY has been reported to suppress growth hormone-releasing hormone secretion (27) and to stimulate somatostatin release. Both mechanisms may result in a consecutive rise in GH activity. A direct or indirect stimulatory effect of GH on leptin production may also theoretically

explain our findings, but appears to be unlikely, since replacement of GH in GH-deficient adults rather results in lower plasma leptin levels due to the accompanying decrease in body fat mass (28).

Our data show that the association between circulating leptin and the IGF system is limited to BMI values below 25 kg/m². Thus, even moderate overweight appears to be associated with the impairment of some regulatory functions of body composition in elderly people. Since the precise mechanism for the interaction between leptin and the IGFs in lean persons is not known, the underlying mechanisms for the lack of correlation in obese individuals is open to speculation. Moreover, measurements of body fat mass were not available in our study, and it is possible that different findings may have been obtained by stratification for fat mass instead of BMI or WHR. However, diet-induced obesity is known to diminish the effects of leptin on weight loss in mice (29), suggesting that leptin may not function properly in obese individuals.

In conclusion, our study shows a significant relationship between serum leptin concentrations and the three major circulating IGF components in lean elderly subjects. The precise mechanism and the overall relevance of this interaction between leptin and the GH/IGF system for body composition remain to be determined.

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